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Development and evaluation of transdermal patches of Colchicine

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ABSTRACT

The purpose of this research was to develop and evaluate transdermal therapeutic system of drug Colchicine with different concentration of drug and hydrophobic (ethyl cellulose) polymeric system by the solvent evaporation technique by using di-butyl phthalate to the polymer weight incorporated as plasticizer. The physiochemical compatibility of the drug and polymer was studied by thin layer chromatography method. Formulated transdermal patches were physically evaluated with regard to thickness, weight variation, drug content, flatness, folding endurance, percentage moisture content, percentage moisture loss and water vapour transmission rate. All prepared formulations indicated good physical stability. In vitro permeation studies of formulations were performed by using skin membrane. The release profile of optimized formulation indicated that the permeation of the drug from the patches was governed by a diffusion mechanism. All formulations showed high flux. These results indicate that formulations gives better permeation of Colchicine.

Key words: Colchicine, transdermal patch, drug content, *in vitro* permeation study.

INTRODUCTION

One goal of controlled-release pharmaceutical dosage forms is to establish relatively constant plasma drug concentration, avoiding the peaks and valleys associated with intermittent dosage form. Transdermal drug delivery systems (TDDS) are dosage forms designed to deliver a therapeutically effective amount of drug across a patient's skin. In order to deliver therapeutic agents through the human skin for systemic effects, the

comprehensive morphological, biophysical and physicochemical properties of the skin are to be considered [1-2].

US FDA approved the first transdermal patch in 1981. This patch delivered scopolamine, a drug which suppresses nausea and vomiting in motion sickness [3]. Treatment of chronic disease such as asthma, rheumatoid arthritis by transdermal route of drug administration might prove to have several advantages over the other routes of administration over the last two decades; more than 35 transdermal products have been approved [4]. This rapid increase in market value has led to transdermal drug delivery becoming one of the fastest growing sectors within pharmaceutical industry [5].

The skin is one of the most extensive and readily accessible organs of the human body. It covers an area of about 2 m^2 and at any point in time is in contact with about one third of all blood circulating through the body [6]. Skin consists of three layers: Epidermis, Dermis and Hypodermis (Subcutaneous tissue). The rate limiting step in percutaneous absorption of most drugs appears to be passage through the stratum corneum [7-9].

In the past topically applied dermatological drugs were used for localized treatment of skin disease only. Recently due to a better understanding of the anatomy and physiology of the skin as well as a more thorough understanding of percutaneous absorption, the limited permeability of human skin has also been utilized for systemic drug administration [10].

Colchicine is obtained from the fully developed dried whole corns, collected before flowering. The colchicine has a specific clinical effect in the treatment of acute gout. Colchicine can be administered both orally and intravenously. Its anticancer properties are attributed to its ability to bind itself to tubulin, the protein subunit of microtubules. It also has anti mitotic activity [11]. Colchicine [*N*-(5, 5, 7, 9-Tetrahydro-1, 2, 3, 10-tetramethoxy-9- oxobenzo [alpha] heptalen-7-yl) acetamide.**]** [12-13**]** occur in the form of pale yellow colour, amorphous powder. It is freely soluble in water (45 mg/ml), chloroform, and benzene (10 mg/ml). Colchicine is slightly soluble in ether (4.5 mg/ml). It having 399.5 dalton molecular weight and melts at about 145°C. To bypass the first pass hepatic metabolism which degrades the potency of the molecule, transdermal patch of Colchicine incorporated with polymer base were prepared to facilitate the drug release, has been formulated [14].

MATERIALS AND METHODS

Materials

Colchicine (Yucca enterprises, Mumbai), Chloroform, Di butyl phthalate, Ethyl cellulose, Poly ethylene Glycol 6000 etc. all chemicals are purchased by CDH lab.

Equipments

UV Visible Spectrophotometer SHIMADZU UV- 1700 PC, Shimadzu Corporation, Japan, Thermostatic hot air oven, Single pan electrical balance- Dhona- 100 DS, pH Strip, Magnetic stirrers and Magnetic Beads, Vacuum Desiccators, Glass beakers, Measuring Cylinders, Graduated pipettes, Funnels, Separating funnels Volumetric flasks, Filter papers, Scissors, Forceps, Clamps and Stands, Tripod Stands, Butter papers, Test tubes, Glass plates, Glass rods, TLC chamber, Surgical blades, Surgical gloves.

Solubility determination

An excess amount of drug was taken and dissolved in measured amount of solvent system having distilled water in to glass vials to get saturated solutions. The solution was kept at rest for 24 hours to assist the attainment of equilibrium with the dissolving drug particles. From these solutions, the supernatant was filtered to separate the undissolved drug particle and diluted suitably and the concentration was measured in UV spectrophotometer. Same procedures were followed for both drug and data for each and every experiment was obtained in triplicate and statically analyzed.

Partition coefficient

The octanol- phosphate buffer pH 7.4 partition was measure of the relative lipophilicity nature of a compound. The partition coefficient of the drugs was determined by shaking equal volume of octanol and phosphate buffer pH 7.4 (aqueous phase) in a separating funnel for 10 minutes and allow to stand for 24 hours. The aqueous phase was assayed before and after partitioning to get the partition coefficients. Similarly skin/vehicle partition coefficients were determined by using skin instead of octanol. Data for each and every experiment was obtained in triplicate and statistically analyzed [15].

Preparation of pig skin

From a Kareli abattoir, ear was obtained from freshly slaughtered pigs. The skin was removed carefully from the outer region of the ear and separated from the underlying cartilage with a scalpel. After separating the full thickness skin, the fat adhering to the dermis side was removed using a scalpel and isopropyl alcohol. Finally the skin was washed with tap water and stored at refrigerator in aluminum foil packing and was used within two days.

Procedure of passive permeation through pig skin

The *in vitro* permeation studies were conducted using vertical test tube cell having a receptor compartment capacity 50 ml. the excised skin was mounted on the top of test tube with the dermis in contact with receptor fluid (Phosphate buffer pH 7.4) and was equilibrated for 240 minutes. The receiving chamber had a volume of 50 ml and the area available for diffusion was about 1.13 cm^2. The fluid in the receptor compartment was maintained 37± .5° C. initially the skin membrane was slightly dipped in the receiving chamber. The entire assembly was kept on a magnetic stirrer and the solution in the receiver compartment was stirred continuously using a magnetic bead. The sample solution was withdrawn from receiving chamber at regular time intervals and assayed

using spectrophotometer. The cumulative amount of drug permeated per cm^2 of skin was plotted against time.

Preparation of transdermal patches of Colchicine

Drug in adhesive type patch were prepared using Ethyl cellulose and poly ethylene glycol 6000 in formulations F-1, F-2, F-3 as adhesive polymer. A chloroform and menthol .5% w/v in ethanol was used as the solvent. Di butyl phthalate were used as plasticizer and cross-linker. The optimized patch formulation was used for further studies. Ethyl cellulose and poly ethylene glycol 6000 were dissolved in a solvent mixture chloroform and menthol. A known quantity solution of Colchicine was added to each polymeric solution. Under continuous stirring, the solutions were allowed to set in the containers for visual checking of whether the drug was dissolved or not. Patches were prepared using a glass plates. The solution was transferred to a glycerin spread glass plates. The films were dried in a room temperature to evaporate the solvents [16].

Table 1: Formulation details of Colchicine transdermal patch

Ingredients	F1	F2	F3
Colchicine	25 mg	50 mg	100 mg
Ethyl Cellulose	1400 mg	1400 mg	1400 mg
PEG 6000	140 mg	140 mg	140 mg
Dibutyl phthalate	0.7 ml	0.7 ml	0.7 ml
Menthol(0.5% w/v in ethanol)	0.1 ml	0.1 ml	0.1 ml
Chloroform	7.0 ml	7.0 ml	7.0 ml

Thickness of film

The film thicknesses of prepared patch were measured by difference using Vernier calipers. The prepared patch was measured at five different points [17-18].

Weight variation study

The study was carried out on three films obtained from casting solution. The mean weight of the film as well as the deviation from the mean was obtained and the data was recorded. The weight of each patch was taken using single pan Dhona balance with sensitivity of 0.01 mg [19-20].

Folding endurance

Evaluation of folding endurance involves determining the folding capacity of the films subjected to frequent extreme condition of folding. Folding endurance was determined by repeatedly folding the film at the same place until it broke. The number of times the film could be folded at the same place without breaking was the folding endurance [21].

Percentage flatness

A transdermal patch should possess a smooth surface and should not constrict with time. This can be demonstrated with flatness study. For flatness determination, one strip is cut from the centre and two from each side of patches. The length of each strip was

measured and variation in length was measured by determining % flatness. Zero percent constriction is equivalent to 100% flatness.

$$\% \text{ constriction} = I_1 - I_2 / I_1 \times 100$$

Where,

I_1= Initial length of each strip,

I_2= Final length of each strip [22, 23].

Percentage moisture content

The prepared patches were cut into 20 X 20 mm strips, were weighed individually and kept in a desiccators containing silica indicative type (Blue) at room temperature for 24 h. The films were reweighed individually until a constant weight was obtained. Percentage of moisture content was then calculated based on the change in the weight with respect to the initial weight of the film.

$$\% \text{ Moisture content} = X - Y / Y \times 100$$

Where,

X = Initial weight

Y = Final weight [24, 25].

Percentage moisture uptake

The water absorption capacities of various films were determined at 82% relative humidity (RH). Films were cut into 20 X 20 mm strips, were weighed, kept in desiccators at room temperature for 24 h, removed and exposed to RH conditions of 82% (containing saturated solution of potassium chloride) in different desiccators at room temperature. Weight was taken periodically until a constant weight was obtained. The water absorption capacity of the films (in weight %) was calculated in terms of percentage increase in the weight of film over the initial weight of the strip.

$$\% \text{ Moisture uptake} = Y - X / Y \times 100$$

Where,

X = Initial Weight

Y = Final Weight [22, 24, 26].

Water vapour transmission rate

Glass vial of equal diameter were used as transmission cells. These transmission cells were washed thoroughly and dried in an oven. The prepared film was fixed over the edge of the glass vial containing 3 gm of fused calcium chloride as a desiccant by using an adhesive. Then the vial was placed in a desiccator containing saturated solution of potassium chloride. The vial was taken out periodically and weighed for a period of 72 h. The experimental was performed in triplicate and the average values were calculated and given result.

$$WVT = WL/S$$

Where,

W = Water vapour transmitting in gm.

L = Thickness of the patch in cm.
S = Exposed surface area in cm. [27-28].

Drug interaction study

Thin layer chromatography was performed to assess any interaction between the drug and the excipients. The solvent system consisted ethyl acetate – methanol – water (100:13.5:10 v/v/v). The data was obtained calculated in terms of R_f value [29-30].

Drug content uniformity

A film of size 10 mm × 10 mm was cut into small pieces and put in a 50 ml phosphate buffer saline 7.4 pH. This was then shaken in a mechanical shaker for 2 hours to get a homogenous solution and filtered. Then appropriate dilution was done. Data for each and every experiment was obtained in triplicate and statistically analyzed. The drug was determined spectroscopically at 350 nm after suitable dilution [24, 31].

In vitro drug permeation of Colchicine transdermal patch through egg membrane

Preparation of Egg membrane:-

From local departmental store EGG was purchase. The skin was removed carefully from the outer region of the egg and separated from the underlying membrane. The outer skin of egg was removed with the help of 0.1 N HCl with constant stirring. After separating the full membrane, the membrane was washed with using phosphate buffer ph 7.4. The membrane was now able to use for further experimental work.

Procedure of Drug Diffusion through Egg Membrane:

The *in vitro* permeation studies were conducted using vertical test tube cell having a receptor compartment capacity 50 ml. The excised egg membrane was mounted with the prepared patch on the top of test tube with the membrane in contact with receptor fluid (Phosphate buffer pH 7.4) and was equilibrated for 210 minutes. The receiving chamber had a volume of 50 ml and the area available for diffusion was about 1.13 cm^2. The fluid in the receptor compartment was maintained 37± .5° C. initially the egg membrane was slightly dipped in the receiving chamber. The entire assembly was kept on a magnetic stirrer and the solution in the receiver compartment was stirred continuously using a magnetic bead. The sample solution was withdrawn from receiving chamber at regular time intervals and replaced by equal volumes of fresh receptor medium and assayed using spectrophotometer. The concentration of colchicine permeated was determined spectrophotometrically at 350 nm after suitable dilution against blank of phosphate buffer saline 7.4 pH by U. V. spectrophotometer. The cumulative amount of drug permeated per cm^2 of skin was plotted against time [32-33].

RESULTS AND DISCUSSION

The present study is to develop and evaluate transdermal patch of Colchicine using polymer as ethyl cellulose and PEG 6000 as plasticizer. The prepared formulation were evaluated for different physiochemical characteristics such as thickness study, folding

endurance, content uniformity, % moisture content, % moisture loss, flatness, weight variation. The release characteristics of formulations were studied in *in vitro* conditions. In vitro permeation studies were carried out in phosphate buffer saline pH 7.4. in order to find out the order of release and the mechanism which predominately influence of the drug release from the membrane. The slope values and the degree of linearity of graphical treatments were considered as important statistical parameters to interpret the *in vitro* profile of all formulations. All the formulations show better results for physiochemical evaluation and *in vitro* release.

Colchicine was scanned in the UV wavelength region of 200-500 nm for maximum absorption (λ max). The λ max was found to be at 350 nm that were same as reported value. (Fig1.) Linear relationship was observed between the concentration and absorbance value in the range of 5 to 30 µg/ml. (Slope=0.0391, R^2=0.9925)

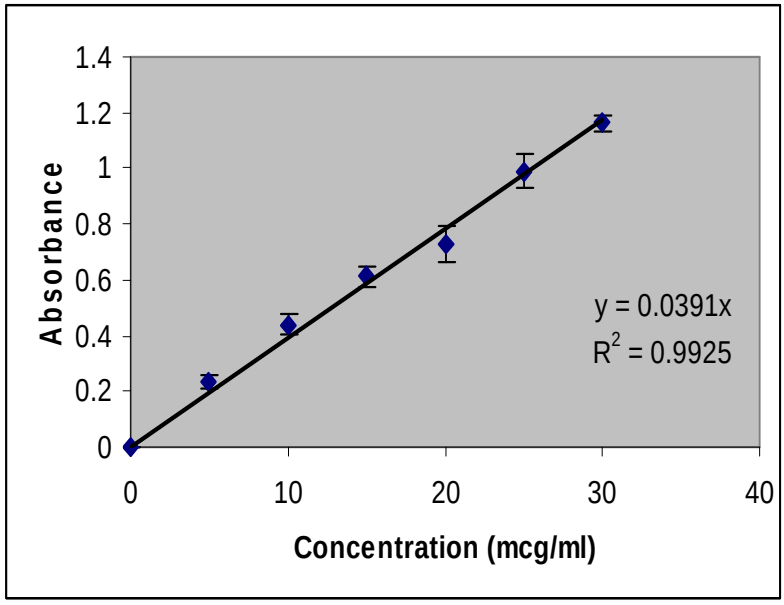

Fig. No. 1. Calibration curve for Colchicine in phosphate buffer saline pH 7.4

Table No. 2. Solubility and partition coefficient of colchicine transdermal patch:

Parameters	Trial 1	Trial 2	Trial 3	Mean ± S.D.
Solubility mg/ml (in dist. water)	41.18	36.64	42.19	40.00±2.956
Partition coefficient (Octanol : Phosphate buffer)	0.80	0.94	0.81	0.85±0.064

Table No. 3. Thickness study of Colchicine transdermal patch

Formulation	Thickness (mm.)						Average± S.D.
	Sample No. 1	Sample No.2	Sample No.3	Sample No.4	Sample No.5	Sample No.6	
F1	0.0200	0.0191	0.0186	0.0198	0.0200	0.0218	0.0198 ± 0.0010
F2	0.0200	0.0196	0.0200	0.0186	0.0189	0.0200	0.0195 ± 0.0006
F3	0.0200	0.0188	0.0216	0.0181	0.0209	0.0198	0.0198 ± 0.0012

Table No. 4. Weight variation study of Colchicine transdermal patch

Formulation	Weight variation (mg.)						Average± S.D.
	Sample No. 1	Sample No.2	Sample No.3	Sample No.4	Sample No.5	Sample No.6	
F1	57	54	59	56	58	57	56.833 ± 1.772
F2	91	87	95	94	88	90	90.833 ± 3.188
F3	148	144	152	146	150	149	148.167 ± 2.857

Table No. 5. Flatness study of Colchicine transdermal patch

Formulation Code	Initial length (cm.)				Final length (cm.)				% Constriction	% Flatness
	Trial 1	Trial 2	Trial 3	Avg.	Trial 1	Trial 2	Trial 3	Avg.		
F1	12.0	3.0	6.5	7.17	12.0	3.0	6.5	7.17	0	100
F2	11.0	4.0	5.8	6.93	11.0	4.0	5.8	6.93	0	100
F3	10.5	2.5	7.1	6.7	10.5	2.5	7.1	6.7	0	100

Table No. 6. Folding endurance study of Colchicine transdermal patch

Formulation code	Folding endurance			Average ± S.D.
	Trial 1	Trial 2	Trial 3	
F1	34	35	36	35 ± 1.000
F2	36	37	36	36 ± 0.577
F3	35	38	35	36 ± 1.732

Table No. 7. Moisture uptake study of Colchicine transdermal patch

Formulation code	Day 1 weight (mg.)				Day 2 weight (mg.)				% Moisture uptake
	Trial1	Trial2	Trial3	Avg. weight	Trial1	Trial2	Trial3	Avg. weight	
F1	58	57	57	57.33	87	84	83	84.66	47.67
F2	91	89	89	89.66	96	94	93	94.33	5.20
F3	148	144	144	145.33	156	153	154	154.33	6.19

Table No. 8. Moisture content study of Colchicine transdermal patch

Formulation code	Day 1 weight (mg.)			Day 2 weight (mg.)			Day 3 weight (mg.)			% Moisture content
	Trial1	Trial2	Trial3	Trial1	Trial2	Trial3	Trial1	Trial2	Trial3	
F1	58	57	58	56	55	57	56	55	57	2.99
F2	91	90	91	91	88	89	91	88	89	1.50
F3	148	146	145	146	144	144	146	144	144	1.15

Table No. 9. Drug excipient interaction study

Sample No.	R_f value for Patch	R_f value of Colchicine std. drug	Interaction
F1	0.62	0.61	Not found
F2	0.62	0.61	Not found
F3	0.61	0.61	Not found

Table No. 10. Water vapour transmission rate study

Batch No.	Water vapour transmitted in (gm)	Water vapour transmission rate (mg/ cm^2)
F1	1.66	0.873
F2	1.55	0.815
F3	1.67	0.878

Table No. 11. Drug content uniformity study

Batch code	Content uniformity %			Drug content uniformity %
	Trial 1	Trial 2	Trial 3	
F1	97.56	98.52	96.52	97.53
F2	98.21	94.36	97.58	96.71
F3	96.48	97.39	98.36	97.41

Table No. 12. Cumulative permeation profile of Colchicine patch in phosphate buffer saline 7.4 pH for formulation 1

Time (min.)	Cumulative permeation (mg/cm^2)			Cumulative release (mg.) ± S.D.
	Trial 1	Trial 2	Trial 3	
15	0.265	0.242	0.282	0.263±0.0200
30	0.302	0.275	0.318	0.298±0.0214
60	0.365	0.351	0.394	0.370 ±0.0219
90	0.392	0.404	0.377	0.391±0.0135
120	0.398	0.411	0.376	0.395±0.0176
150	0.336	0.347	0.242	0.308±0.0577
180	0.354	0.434	0.24	0.342±0.0974
210	0.435	0.46	0.266	0.387±0.1055

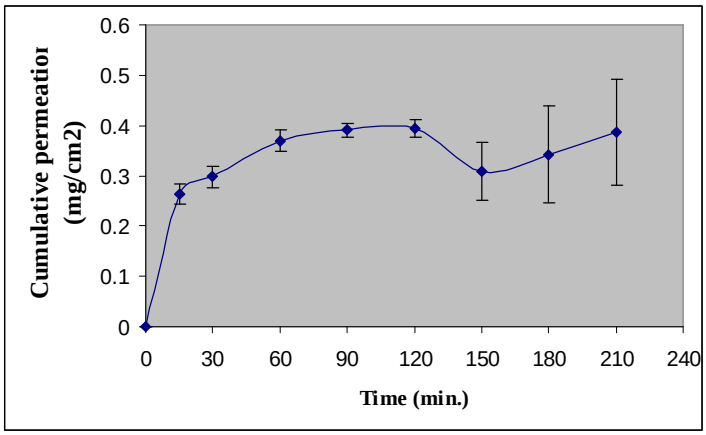

Fig. No. 2. Cumulative permeation profile of Colchicine patch in phosphate buffer saline 7.4 pH for formulation 1

Table No. 13. Cumulative permeation profile of Colchicine patch in phosphate buffer saline 7.4 pH for formulation 2

Time (min.)	Cumulative permeation (mg/cm^2)			Cumulative release (mg.) ± S.D.
	Trial 1	Trial 2	Trial 3	
15	0.361	0.394	0.315	0.356 ± 0.0396
30	0.315	0.278	0.329	0.307 ± 0.0263
60	0.322	0.269	0.350	0.313 ± 0.0411
90	0.266	0.297	0.263	0.275 ± 0.0188
120	0.407	0.391	0.418	0.405 ± 0.0135
150	0.531	0.595	0.517	0.547 ± 0.0415
180	0.390	0.418	0.330	0.379 ± 0.0449
210	0.310	0.317	0.286	0.304 ± 0.0162

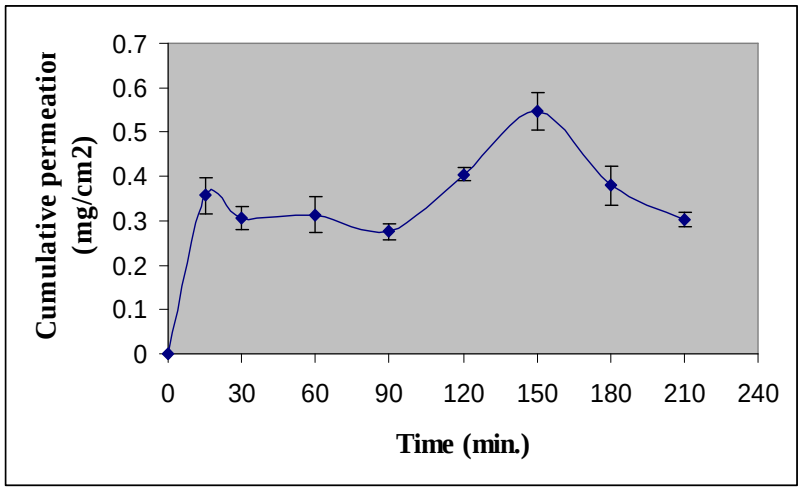

Fig No. 3. Cumulative permeation profile of Colchicine patch in phosphate buffer saline 7.4 pH for formulation 2

Table No. 14. Cumulative permeation profile of Colchicine patch in phosphate buffer saline 7.4 pH for formulation 3

Time (min.)	Cumulative permeation (mg/cm^2)			Cumulative release (mg.) ± S.D.
	Trial 1	Trial 2	Trial 3	
15	0.318	0.312	0.335	0.321 ± 0.0119
30	0.363	0.297	0.400	0.353 ± 0.0521
60	0.398	0.327	0.440	0.388 ± 0.0571
90	0.451	0.375	0.531	0.452 ± 0.0780
120	0.500	0.385	0.527	0.470 ± 0.0754
150	0.347	0.216	0.374	0.312 ± 0.0845
180	0.423	0.282	0.466	0.390 ± 0.0962
210	0.354	0.296	0.419	0.356 ± 0.0615

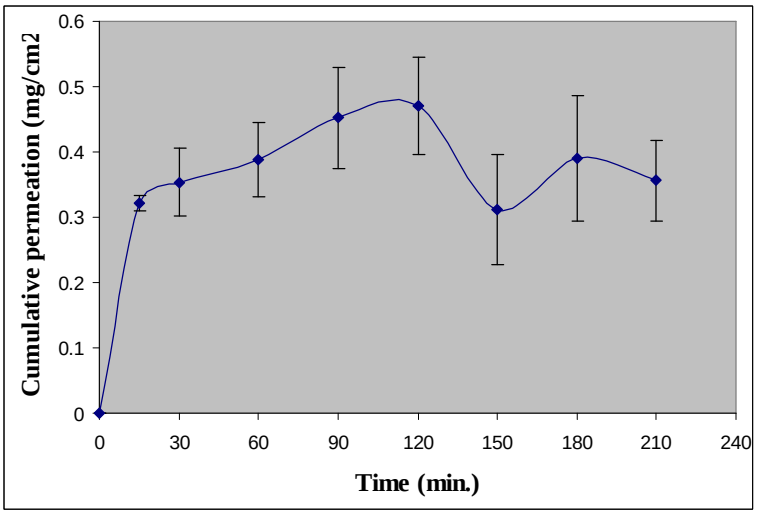

Fig No. 4. Cumulative permeation profile of Colchicine patch in phosphate buffer saline 7.4 pH for formulation 3

Table No. 15. Steady state flux, permeability and diffusion coefficient of different formulation of colchicine patches

Formulation code	Steady state flux (mg/h/cm)	Diffusion coefficient (cm^2/s)	Permeability coefficient (cm/h)
F1	0.025	1.400×10^{-3}	0.0595
F2	0.092	0.9741×10^{-3}	0.0414
F3	0.099	0.6918×10^{-3}	0.0294

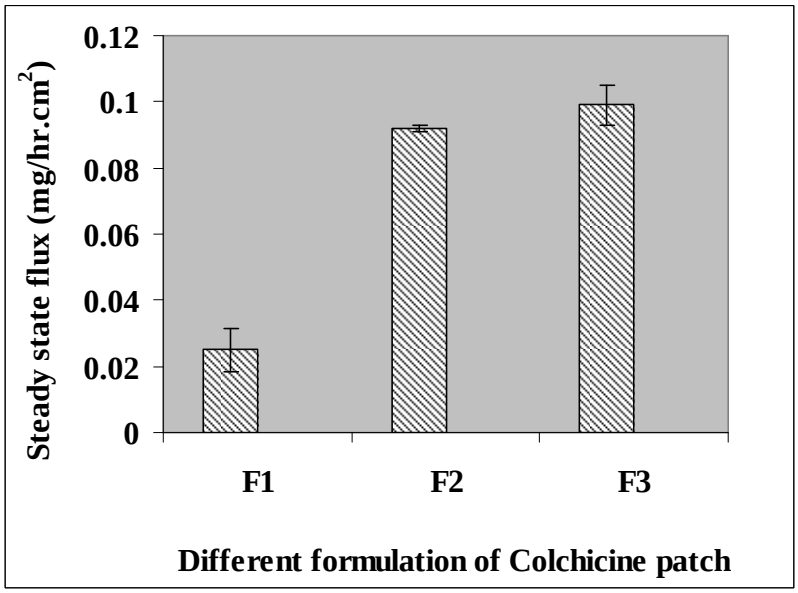

Fig. No. 5. Comparision of steady state flux between different formulations of Colchicine patch

The solubility of drug Colchicine was determined in water. The solubility studies indicate that the drug is freely soluble in water and very soluble in chloroform and benzene. (Table No. 2.) The hydrophilic and hydrophobic nature was again established by performing Octanol : Phosphate buffer saline 7.4 pH, which was found to be 0.85 ±0.0637. This indicates these have the greater affinity towards the lipoidal skin. This partitioning value near to unity is the indicative of hydrophilic and hydrophobic nature of the drug shows that the drug is good candidate for transdermal drug delivery. (Table No. 2.)

The physiochemical evaluations of the formulations have been shown different physical characteristic which change according to the formulations. The film exhibited thickness F1 = 0.0198±0.0010, F2 0.0195 ± 0.0006 and F3 0.0198 ± 0.0012 and weight F1 56.833 ± 1.772, F2 90.833 ± 3.188 and F3 148.167 ± 2.857. (Table No. 3 and 4) The thickness was approximately close to every formulation and weight was found to be maximum for F3 and minimum for F1 due to viscosity variation of polymeric solution used in the polymeric films.

The percentage flatness data of the prepared patch were shown in Table No. 5. It was evident that there was no much deviation in the flatness reveals uniform patches.

The folding endurance of the films varied form 35 ± 1 to 36 ± 1.732 number of fold. From results found that the folding endurance of F3 is greater then F1 and F2. (Table No. 6.)

The higher percentage of moisture uptake and moisture content was found in formulation F1 47.67 and 2.99 respectively which may be due to the hydrophobic nature of polymer used. (Table No. 7. and 8.)

Interaction studies were carried out to ascertain any interaction of the drug with the excipient used in the preparation of TDDS. The drug excipient interaction study was performed with the help of thin layer chromatography Table No. 9. The study found that the every formulation was interaction free. The results indicate that the drug remain intact in TDDS and there was negligible chemical interaction between the drug and the excipient there in.

The WVTR was 0.873 mg/cm^2 for F1, 0.815 mg/cm^2 for F2 and 0.878 mg/cm^2 for F3. Maximum WVTR was seen in F3. (Table No. 9.)

The drug content uniformity of the prepared for formulations have shown in Table No. 11. That the process used to prepare the films in this study was capable of giving films with uniform drug content. The content found to be in F1 = 97.53% was greater then the F2 = 96.71% and F3 = 97.41%.

In vitro skin permeation studies predict *in vivo* performance of drug. It was performed on pig ear skin. The drug release is shown in Fig. No. 2, 3 and 4. and in Table no. 12, 13 and 14. The formulation showed good linearity.

The value of permeation parameters are depicted in Table No. 15 and Fig No. 5. Although steady state flux is the most therapeutically relevant parameter, permeability coefficient are usually used for comparison purpose. It is evident the permeability coefficient decreased with the increase in donor concentration.

CONCLUSION

This study demonstrated that a novel transdermal drug delivery system (TDDS) patch composed of Colchicine. The patches were prepared using ethyl cellulose and PEG 6000 along with various solvent such as chloroform, ethanol and di-butyl phthalate as plasticizer. The obtained patch was thin, flexible, smooth and yellowish in colour. Thickness, weight and drug content of all the formulation remained uniformly with low SD values. Percentage moisture content and Percentage moisture loss was decreased with the higher concentration of Colchicine. Prepared patch was found 0% constriction or 100% flatness with good folding capacity. *In vitro* drug release study through skin membrane of formulation F1, F2 and F3 showed better release pattern and physiochemical characteristics. In conclusion the present data confirm the feasibility of developing Colchicine transdermal patches on an industrial scale. Further studies, now in progress, will deal with the application of the presently reported findings to human skin permeation, involving pharmacodynamic and pharmacokinetic testing.

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